

MARKED-UP VERSION OF AMENDED PARAGRAPHS IN THE SPECIFICATION

In the following amendments, underlining denotes added text while bracketing denotes deleted text. For the Tables, the only changes involve deleting the original shading within some cells and replacing it with text boxes. As these changes are too difficult to show with underlining and bracketing, no underlining or bracketing are used below. Should the Examiner require a different format to indicate these amendments, she is encouraged to contact Applicants.

IN THE SPECIFICATION:

Please replace the paragraph on page 1 under "Cross-Reference to Related Applications" with the following:

Pursuant to 35 U.S.C. §119(e), the present application claims benefit of and priority to US Application No. 60/233,610, entitled "Twin-Arginine Translocation in Bacillus", filed September 18, 2000[1] by Jongbloed et al.

Please replace Table I and the text following the Table, on page 56 with the following:

Table I. Predicted Twin-Arginine Signal Peptides of *B. subtilis**

protein	signal peptide
AlbB	SPAQRRLILYL ILSFIFVIGAVVYFVKSDYLFTLIFI AIAILF
AmyX TM	MVSIRRSFE AYVDDMNIIITVLIPAEQKEIM
AppB TM	MAAYIIIRRT LMSIPIILLGITILSFVIM KAAPG
LipA	MKFVKRR IIALVTILMLSVTSLFAL QPSAKAAEH
OppB TM	MLKYIGRR LVYMIITLFVIVTVTFFLM QAAPG
PbpX	MTSPTRRRTAKRRRRKLNKR GKLLFGLLAVMVCITI WNALHR
PhoD	MAYDSRFDEWVQKLKEESFQNNTFDRRKFIQ GAGKIAGLSLGLTIAQS VGAFEV
QcrA	MGGKHDISRRQFLN YTLTGVGGFMAASMLMPMV RFA
SpoIIIJ TM	MLLKRR IGLLLLSMVGVFML LAGCSSV
TlpA TM	MKKTLTTIIRRSSIARR LIISFLLILIVPITALSVSAY QS
WapA	MKKRKRRNFKRFIAAFLVLALMISLVPAD VLA KST
WprA	KRRKFSS VVAAVLIFALIFSLFSPG TKAAAAGA
YceA TM	MEMFDLEFMRR AFLAGGMIAVMAPILGVVLV RRQ
YdeJ	MKKRRK ICYCNTALLLMIL LAGCTDS
YdhF	MRR ILSILVFAIM LAGCSSN
YdhK	MSAGKSYRKKM QRRMN MKISK YALGILMLSLV FVL SA CGNNN
YesM TM	KKRVAGWYRRMKIKDK LFVFLSLIMAVSFLFVYSGV QYAFHV
YesW	MRRSCLMIRR RKRMFTA VL LLVLLVMGTSVCPV KAEGA
YfkN TM	MRIQKRRTHVENILR ILLPPIMILS LILPT PP IHAEES
YkpC	MLRDLGRR VAIAAILSGI ILGGMSI SLANMP
YkuE	MKKMSRRQFLK G MF G ALAAGALT AGGGY GYARYL
YmaC	MRRFLLN V ILVLAIVLFLRYV HYSLEPE
YmzC	MFESEAE LRR IRIALVWIAVFL L FGA C GN
YubF TM	MQKYRRRN T VAFTV L AYFTFFAGVFLFSIGLY N ADNL
YuiC	MMLN M IRRL L MTCL F LLA F GTT F LSVSG I EAKDL
YvhJ	MAERVRVRVRKKKSKRRKILKR IMLLF ALALLVV V GLGGY K LY
YwbN	MSDEQKKPEQI HRR DILK W GAMAGAAVAIGASGLGG L PLV Q TAAKP

* Putative twin-arginine signal peptides were identified in two ways. First, the presence of the

consensus sequence R-R-X- ϕ - ϕ (ϕ is a hydrophobic residue), immediately in front of an amino-terminal hydrophobic region as predicted with the TopPred2 algorithm (34, 35), was determined. To this purpose, the first 60 residues of all annotated proteins of *B. subtilis* in the SubtiList database (<http://bioweb.pasteur.fr/Genolist/Subtilist.html>) were used. Second, within the group of twin-arginine membrane sorting signals, cleavable signal peptides were identified with the SignalP algorithm (61, 62). Conserved residues of the twin-arginine consensus sequence (R-R-X- ϕ - ϕ) are indicated in bold. In addition, positively charged residues that could function as a so-called Sec-avoidance signal (54) are indicated in bold and italics. The hydrophobic H-domain is indicated ~~in gray shading~~ in boxed text. In signal peptides with a predicted signal peptidase I cleavage site, residues from position -3 to -1 relative to the signal peptidase I cleavage site are underlined. Notably, some of these proteins contain one or more putative transmembrane segments elsewhere in the protein (indicated with "TM"), or are putative lipoproteins. Residues forming a so-called lipobox for signal peptidase II cleavage are enlarged in size.

Please replace Table IV and following text, on page 59, with the following:

Table IV. Twin-Arginine Signal Peptides of PhoD and PhoD-like proteins*

protein	signal peptide
PhoD (<i>Bsu</i>)	MAYDSRFDEWVQKLKEESFQNNTFDRRKFI <u>Q</u> GAGKIAGLSLGLTIAQS VGAFEV
SP1 (<i>Sco</i>)	MTPANHQAPTSAPSPAPSQSSHAPELRAAARSLGRRRFLT <u>VTGAAAALAFAVNLPAAG</u> TA <u>SAAEL</u>
SP2 (<i>Sco</i>)	MAPTGRPSALAEHAFSPHDAVLGAAARHLGRRRFLT <u>VTAAAAALAFSTNL</u> PARGAVAAPE
SP3 (<i>Sco</i>)	MTSRHRASENSRTPSRRTVVK <u>AAAAGAVLAAPLAAALPAGA</u> ADAAPA
SP4 (<i>Ste</i>)	MTPAARPSQHAPELRAAARHLGRRRFLT <u>VTGAAAALAFAVNLPAAG</u> TAAAEL

* Homologues of *B. subtilis* PhoD were identified by amino acid sequence similarity searches in GenBank using the BLAST algorithm. SP1 (*Sco*), gene SCC75A.32c of *Streptomyces coelicolor* (CAB61732); SP2 (*Sco*), gene SCF43A.18 of *S. coelicolor* (CAB48905); SP3 (*Sco*), gene SC4G6.37 of *S. coelicolor* (CAB51460), and SP4, *phoD* gene of *Streptomyces tendae*

(CAB62565). GenBank accession numbers are indicated in parentheses. Conserved residues of the twin-arginine consensus sequence are indicated in bold. The hydrophobic H-region is indicated by boxed text, in gray shading. Signal peptidase I recognition sequences predicted with the SignalP algorithm (61, 62) are underlined.